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(54) **Marek's disease virus vaccine.**

(57) The invention is concerned with the MD18 and MD20 polypeptides of Marek's Disease virus which can be used to vaccinate poultry against MD.

The invention also relates to nucleic acid sequences encoding the MD18 or MD20 polypeptides. Said sequences can be used for the preparation of a subunit or vector vaccine.

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The present invention is concerned with a nucleic acid sequence encoding a Marek's Disease virus polypeptide, a recombinant nucleic acid molecule comprising such a nucleic acid sequence, a vector virus comprising said nucleic acid sequence, a host cell transformed with such a nucleic acid sequence, a Marek's Disease virus polypeptide and antibodies reactive therewith, as well as a vaccine against Marek's Disease.

Marek's Disease (MD) is a malignant, lymphoproliferative disorder of domestic fowl caused by a herpesvirus: Marek's Disease Virus (MDV). MD is ubiquitous, occurring in poultry-producing countries throughout the world. Chickens raised under intensive production systems will inevitably suffer losses from MD. MD affects chickens from about 6 weeks of age, occurring most frequently between ages of 12 and 24 weeks.

Three forms of MD are recognized clinically, classical MD, acute MD and transient paralysis.

Classical MD is characterized by peripheral nerve enlargement caused by lymphoid infiltration and demyelination, and paralysis is the dominant clinical sign. Mortality is variable but normally under 10-15 per cent.

In the acute form there are multiple and diffuse lymphomatous tumours in the visceral organs. Mortality from this form of MD is usually higher than from the classical form. An incidence of 10-30 per cent is common in unvaccinated flocks and outbreaks involving up to 70% of the flock may be encountered. The pathological lesions in both classical and acute MD are essentially similar, involving the proliferation and infiltration of malignantly transformed T-lymphoblasts into normal tissues, peripheral nerves in the case of the classical form and visceral organs in the case of the acute form.

Furthermore, the MDV has been shown to be responsible for encephalitis of young chickens characterized by sudden paralysis.

Serological classification of MD related viruses yielded three serotypes:

Type I : naturally occurring virulent strains of Marek's disease virus which are pathogenic and tumorigenic to chickens, and attenuated nonpathogenic strains derived therefrom

Type II : naturally occurring nonpathogenic strains of Marek's disease virus; and

Type III : herpesvirus of turkeys ("HVT"), which is nonpathogenic to chickens.

Serial passage of pathogenic strains of MDV serotype I was found to result in loss of pathogenicity and oncogenicity, but not of immunogenicity. Attenuated strains derived from HPRS-16 and CVI-988 strains have been applied as vaccines. SB-I and HN-I MDV strains (serotype 2) have also been shown to be useful in vaccination. HUT, first isolated from turkeys, is apathogenic in turkeys and domestic fowls, antigenically related to serotype 1 and 2 MD viruses and extensively used as a vaccine against MD.

There are no methods of treatment of MD and control is based on management methods which isolate growing chickens from sources of infection, the use of genetically resistant stock, and vaccination. However, management procedures are normally not cost-effective and the progress has been disappointing with respect to the selection of poultry stock with increased genetically controlled resistance. Nowadays, control of MD is almost entirely based on vaccination.

Current vaccines comprise chemically inactivated virus vaccines or modified live-virus vaccines. However, inactivated vaccines require additional immunizations, disadvantageously contain adjuvants, are expensive to produce and are laborious to administer. Further, some infectious virus particles may survive the inactivation process and may cause disease after administration to the animal.

In general, attenuated live virus vaccines are preferred because they evoke an immune response often based both humoral and cellular reactions. Up to now, such vaccines based on MDV serotype I strains can only be prepared by serial passage of virulent strains in tissue culture. However, because of this treatment uncontrolled mutations are introduced into the viral genome, resulting in a population of virus particles heterogeneous with regard to virulence and immunizing properties. Overattenuation during passage in cell culture can also be a problem with these vaccines. One must achieve a delicate balance between ensuring that the vaccine is not virulent while making certain that it is still protective. In addition it is well known that such traditional attenuated live virus vaccines can revert to virulence resulting in disease outbreaks in inoculated animals and the possible spread of the pathogen to other animals. The occurrence of very virulent field strains of MD virus against which live HVT vaccines provided poor protection have now been isolated and are responsible for excessive losses in various parts of the world. Bivalent vaccines consisting of serotype 2 and serotype 3 strains are reasonably effective against very virulent field isolates in some cases. Multivalent vaccines containing serotype 1 antigens should be even more effective at eliciting immunity against these very virulent strains.

Improved vaccines might be constructed based on recombinant DNA technology. These vaccines would only contain the necessary and relevant MDV immunogenic material which is capable of eliciting a protective immune response against the MDV pathogens, or the genetic information encoding said material, and would not display above-mentioned disadvantages of the live or inactivated vaccines.

5 According to the present invention a nucleic acid sequence encoding a MDV polypeptide is provided which can be applied for the preparation of a vaccine for the immunization of poultry against MD.

"Nucleic acid sequence" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxy-ribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, as well as  
10 double and single stranded RNA, and modifications thereof.

In general, the term "polypeptide" refers to a molecular chain of amino acids with a biological activity and does not refer to a specific length of the product. If required the polypeptide can be modified in vivo or in vitro, for example by glycosylation, amidation, carboxylation or phosphorylation; thus inter alia, peptides, oligopeptides and proteins are included.

15 According to the invention a nucleic acid sequence containing a gene encoding the MDV polypeptide MD18 or MD20, respectively, have been isolated and characterized and were found to be recognized by the immune system of the host. The genes encoding said polypeptides were identified by screening bacteriophage expression libraries made in the lambda gt11 vector, with polyvalent sera from chickens infected with a virulent MD virus strain.

20 The gene encoding the MD18 polypeptide maps to the unique long ( $U_L$ ) region of the MDV genome and encodes a polypeptide of about 663 amino acids in length. The amino acid sequence of the polypeptide encoded by the MD18 gene is shown in SEQ ID NO: 2.

The gene encoding the MD20 polypeptide also maps to the  $U_L$  region of the MDV genome and encodes a polypeptide of about 1074 amino acids in length. The amino acid sequence of the polypeptide encoded  
25 by the MD20 gene is shown in SEQ ID NO: 4.

More particularly, this invention provides a nucleic acid sequence that encodes the MD18 polypeptide or MD20 polypeptide having an amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, respectively.

Also included within the scope of the present invention are nucleic acid sequences encoding a  
30 functional equivalent of said MD18 or MD20 polypeptide having corresponding immunological characteristics.

It will be understood that for the particular MD18 or MD20 polypeptide embraced herein, derived from the serotype 1 GA strain, natural variations can exist between individual viruses or strains of MDV of type 1. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by  
35 deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions from which can be expected that they do not essentially alter biological and immunological activities have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington  
40 D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the similarity between homologous polypeptides. Nucleic acid sequences encoding such functional equivalents are included within the scope of this invention. Moreover, the potential exist to use recombinant DNA technology for the preparation of nucleic acid sequences encoding these various functional equivalents.

45 Preferably, nucleic acid sequences according to the invention may be derived from available isolates of MDV of serotype 1, strains such as GA, JM, HPRS-16, Conn A, RB-IB CVI-988 or Md 11, the GA strain being the most preferred strain.

In addition nucleic acid sequences encoding the MD18 polypeptide or MD20 polypeptide or variations thereof as mentioned above may also be derived from MDV strains belonging to serotype 2 or serotype 3,  
50 e.g. HN, HPRS-24, SB-1 or FC126.

The information provided in SEQ ID NO: 1-4 allows a person skilled in the art to isolate and identify the nucleic acid sequences encoding the variant functional equivalent polypeptides mentioned-above having corresponding immunological characteristics with the MD18 or MD20 polypeptide disclosed herein. The generally applied blotting and hybridization techniques can be used for that purpose (Experiments in  
55 Molecular Biology, ed. R.J. Slater, Clifton, U.S.A., 1986; Singer-Sam, J. et al., Proc. Natl. Acad. Sci. 80, 802-806, 1983; Maniatis, T. et al., in Molecular Cloning, A laboratory Manual, Cold Spring Harbor Laboratory Press, U.S.A., 1989). For example, restriction enzyme digested DNA fragments derived from a specific MDV strain is electrophoresed and transferred, or "blotted" thereafter onto a piece of nitrocellulose filter. It is now

possible to identify the nucleic sequences encoding the functional equivalent polypeptides on the filter by hybridization to a defined labelled DNA or "probe" back translated from the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, under specific conditions of salt concentration and temperature that allow hybridization of the probe to any homologous DNA sequences present on the filter. After washing the filter, hybridized material may be detected by autoradiography. From an agarose gel with starting DNA that was not blotted, DNA can now be obtained that encodes a polypeptide functionally equivalent to a polypeptide disclosed in SEQ ID NO: 2 or 4.

In another way, DNA obtained from a specific MDV strain may be cloned into a  $\lambda$ gt11 phage and expressed into a bacterial host. Recombinant phages can then be screened with polyclonal serum raised against the purified MD18 or MD20 polypeptide, determining the corresponding immunological characteristic of the variant polypeptide. Above-mentioned procedure is outlined in detail in Example 1. The production of the polyclonal serum elicited against MD18 or MD20 is described below.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon but still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4 use can be made of a derivative nucleic acid sequence (functional equivalent) with such an alternative codon composition different from the nucleic acid sequence shown in said SEQ ID's.

A preferred nucleic acid sequence according to the invention is characterized in that said sequence contains the deoxyribonucleic acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 3.

Furthermore, also fragments of the nucleic acid sequences encoding the MD18 or MD20 polypeptide or functional equivalents thereof as mentioned above are included in the present invention.

The term "fragment" as used herein means a DNA or amino acid sequence comprising a subsequence of one of the nucleic acid sequences or polypeptides of the invention. Said fragment is or encodes a polypeptide having one or more immunoreactive and/or antigenic determinants of a MDV polypeptide, i.e. has one or more epitopes which are capable of eliciting an immune response in a chicken and/or is capable of specifically binding to a complementary antibody. Methods for determining usable polypeptide fragments are outlined below. Fragments can inter alia be produced by enzymatic cleavage of precursor molecules, using restriction endonucleases for the DNA and proteases for the polypeptides. Other methods include chemical synthesis of the fragments or the expression of polypeptide fragments by DNA fragments introduced in a suitable host cell environment.

All modifications resulting in such functional equivalents of the MD18 or MD20 polypeptide are included within the scope of the present invention for as long as the immunological characteristics of the MD18 or MD20 polypeptide remain unaffected in essence.

A nucleic acid sequence according to the present invention can be ligated to various replication effecting DNA sequences with which it is not associated or linked in nature, optionally containing portions of DNA encoding fusion protein sequences such as  $\beta$ -galactosidase, resulting in a so called recombinant nucleic acid molecule which can be used for the transformation of a suitable host. Such hybrid DNA molecules, are preferably derived from, for example plasmids, or from nucleic acid sequences present in bacteriophages, cosmids or viruses. Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art and include inter alia plasmid vectors such as pBR322, the various pUC, pGEM and Bluescript plasmids, bacteriophages, e.g.  $\lambda$ gt-Wes- $\lambda$  B, Charon 28 and the M13 derived phages or viral vectors such as SV40, adenovirus or polyoma virus (see also Rodriguez, R.L. and D.T. Denhardt, ed., Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988; Lenstra, J.A. et al., Arch. Virol. 110, 1-24, 1990). The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Maniatis, T. et al. (ibid, 1989). For example, the insertion of the nucleic acid sequence according to the invention into a cloning vector can easily be achieved by ligation with an enzyme such as T<sub>4</sub> DNA ligase when both the genes and the desired cloning vehicle have been cut with the same restriction enzyme(s) as complementary DNA termini are thereby produced.

Alternatively, it may be necessary to modify the restriction sites that are produced into blunt ends either by digesting the single-stranded DNA or by filling in the recessive termini with an appropriate DNA polymerase. Subsequently, blunt end ligation with an enzyme such as T<sub>4</sub> DNA ligase may be carried out.

If desired, any restriction site may be produced by ligating linkers onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site sequences. The restriction enzyme cleaved vector and nucleic acid sequence may also be modified by homopolymeric tailing.

"Transformation", as used herein, refers to the introduction of a heterologous nucleic acid sequence into a host cell, irrespective of the method used, for example direct uptake or transduction. The heterologous nucleic acid sequence may be maintained through autonomous replication or alternatively, may be integrated into the host genome. If desired, the recombinant DNA molecules are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted nucleic acid sequence.

The recombinant nucleic acid molecule according to the invention preferably contains one or more marker activities that may be used to select for desired transformants, such as ampicillin and tetracycline resistance in pBR322, ampicillin resistance and  $\beta$ -galactosidase activity in pUC8.

A suitable host cell is a cell which can be transformed by a nucleic acid sequence encoding a polypeptide or by a vector virus or a recombinant nucleic acid molecule comprising such a nucleic acid sequence and which can if desired be used to express said polypeptide encoded by said nucleic acid sequence. The host cell can be of procaryotic origin, e.g. bacteria such as *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas* species; or of eucaryotic origin such as yeast, e.g. *Saccharomyces cerevisiae* or higher eucaryotic cells such as insect, plant or mammalian cells, including HeLa cells and Chinese hamster ovary (CHO) cells. Insect cells include the Sf9 or Sf21 cell line of *Spodoptera frugiperda* (Luckow et al., *Biotechnology* 6, 47-55, 1989). Information with respect to the cloning and expression of the nucleic acid sequence of the present invention in eucaryotic cloning systems can be found in Esser, K. et al. (Plasmids of Eukaryotes, Springer-Verlag, 1986).

In general, prokaryotes are preferred for cloning and manipulation of DNA sequences and for constructing the vectors useful in the invention. For example *E. coli* K12 is particularly useful. Other microbial strains which may be used include *E. coli* strains such as DH5 $\alpha$ , JM101 or HB101.

For expression, nucleic acid sequences of the present invention are operably linked to expression control sequences. Such control sequences may comprise promoters, enhancers, operators, inducers, ribosome binding sites etc.

When the host cells are bacteria, illustrative useful expression control sequences include the Trp promoter and operator (Goeddel, et al., *Nucl. Acids Res.* 8, 4057, 1980); the lac promoter and operator (Chang, et al., *Nature* 275, 615, 1978); the outer membrane protein (OMP) promoter (Nakamura, K. and Inouge, M., *EMBO J.* 1, 771-775, 1982); the bacteriophage  $\lambda$  promoters and operators (Remaut, E. et al., *Nucl. Acids Res.* 11, 4677-4688, 1983); the  $\alpha$ -amylase (*B. subtilis*) promoter and operator, termination sequence and other expression enhancement and control sequences compatible with the selected host cell. When the host cell is yeast, illustrative useful expression control sequences include, e.g.,  $\alpha$ -mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G.E. et al., *Mol. Cell. Biol.* 3, 2156-65, 1983). When the host cell is of mammalian origin illustrative useful expression control sequences include, e.g., the SV-40 promoter (Berman, P.W. et al., *Science* 222, 524-527, 1983) or, e.g. the metallothionein promoter (Brinster, R.L., *Nature* 296, 39-42, 1982) or a heat shock promoter (Voellmy et al., *Proc. Natl. Acad. Sci. USA* 82, 4949-53, 1985). Alternatively, also expression control sequences present in MDV, in particular those regulating the expression of MD18 or MD20 may be applied. For maximizing gene expression, see also Roberts and Lauer (*Methods in Enzymology* 68, 473, 1979).

The present invention also comprises a polypeptide displaying MDV immunological characteristics containing at least part of the MD18 or MD20 amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, respectively, or derivatives thereof, essentially free from the whole virus or other protein with which it is ordinarily associated.

It will be understood that derivatives of said amino acid sequences displaying the same immunological properties in essence, i.e. immunological equivalents, are also within the scope of the present invention.

Immunological equivalents of the MD18 or MD20 polypeptide disclosed herein are the corresponding polypeptides present in viruses of other strains of MD serotype 1 or in viruses of strains belonging to serotype 2 or 3. Said equivalents can be produced through the expression of the genes encoding said equivalents, the genes being identified and isolated making use of the information provided herein as described above.

In addition a polypeptide comprising a fragment of the MD18 or MD20 polypeptide or functional equivalent thereof, which can be used for immunization of poultry against MD is included in the present invention. Various methods are known for detecting such usable polypeptide fragments within a known amino acid sequence.

Suitable immunochemically active polypeptide fragments of a polypeptide according to the invention containing (an) epitope(s) can be found by means of the method described in Patent Application WO 86/06487, Geysen, H.M. et al. (*Prod. Natl. Acad. Sci.* 81, 3998-4002, 1984), Geysen, H.M. et al. (*J. Immunol.*

Meth. 102, 259-274, 1987) based on the so-called pep-scan method, wherein a series of partially overlapping polypeptides corresponding with partial sequences of the complete polypeptide under consideration, are synthesized and their reactivity with antibodies is investigated.

In addition, a number of regions of the polypeptide, with the stated amino acid sequence, can be designated epitopes on the basis of theoretical considerations and structural agreement with epitopes which are now known.

The determination of these regions was based on a combination of the hydrophilicity criteria according to Hopp and Woods (Proc. Natl. Acad. Sci. 78, 3824-3828, 1981) and the secondary structure aspects according to Chou and Fasman (Advances in Enzymology 47, 45-148, 1987).

T-cell epitopes which may be necessary can likewise be derived on theoretical grounds with the aid of Berzofsky's amphiphilicity criterion (Science 235, 1059-62, 1987).

Such epitopes can also be generated experimentally by limited exposure of the polypeptide to the proteolytic activity of Cathepsin D. Cleavage of a protein antigen by this enzyme specifically recognizes amino acid sequence patterns which are also found within the NH<sub>2</sub>-terminal residues of peptides recognized by the major histocompatibility complex on the surface of antigen-presenting cells (v. Noort, J.M. and v.d. Drift, A.C.M., J. Biol. Chem. 264, 14159, 1989).

In another embodiment of the invention a polypeptide having an amino acid sequence encoded by a nucleic acid sequence mentioned above is used.

Immunization of poultry against MDV infection can, for example be achieved by administering to the animals a polypeptide according to the invention in an immunologically relevant context as a so-called subunit vaccine. The subunit vaccine according to the invention may comprise a polypeptide in a pure form, optionally in the presence of a pharmaceutically acceptable carrier. The polypeptide can optionally be covalently bonded to a non-related protein, which, for example can be of advantage in the purification of the fusion product. Examples are  $\beta$ -galactosidase, protein A, prochymosin, blood clotting factor Xa, etc.

In some cases the ability to raise neutralizing antibodies against these polypeptides per se may be low. Small fragments are preferably conjugated to carrier molecules in order to increase their immunogenicity. Suitable carriers for this purpose are macromolecules, such as natural polymers (proteins like keyhole limpet hemocyanin, albumin, toxins), synthetic polymers like polyamino acids (polylysine, polyalanine), or micelles of amphiphilic compounds like saponins. Alternatively these fragments may be provided as polymers thereof, preferably linear polymers.

Polypeptides to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolating said polypeptides from MDV, by recombinant DNA techniques or by chemical synthesis.

If required the polypeptides according to the invention to be used in a vaccine can be modified in vitro or in vivo, for example by glycosylation, amidation, carboxylation or phosphorylation.

An alternative to subunit vaccines are live vector vaccines. A nucleic acid sequence according to the invention is introduced by recombinant DNA techniques into a micro-organism (e.g. a bacterium or virus) in such a way that the recombinant micro-organism is still able to replicate thereby expressing a polypeptide coded by the inserted nucleic acid sequence.

For example the technique of in vivo homologous recombination can be used to introduce a heterologous nucleic acid sequence, e.g. a nucleic acid sequence according to the invention into the genome of the vector micro-organism.

First, a DNA fragment corresponding with an insertion region of the vector genome, i.e. a region which can be used for the incorporation of a heterologous sequence without disrupting essential functions of the vector such as those necessary for infection or replication, is inserted into a cloning vector according to standard recDNA techniques. Insertion-regions have been reported for a large number of micro-organisms (e.g. EP 80,806, EP 110,385, EP 83,286, EP 314,569, WO 88/02022 and WO 88/07088).

Second, if desired, a deletion can be introduced into the insertion region present in the recombinant DNA molecule obtained from the first step. This can be achieved for example by appropriate exonuclease III digestion or restriction enzyme treatment of the recombinant DNA molecule from the first step.

Third, the heterologous nucleic acid sequence is inserted into the insertion-region present in the recombinant DNA molecule of the first step or in place of the DNA deleted from said recombinant DNA molecule. The insertion-region DNA sequence should be of appropriate length as to allow homologous recombination with the vector genome to occur. Thereafter, suitable cells can be transformed with vector genomic DNA in the presence of the recombinant DNA molecule containing the insertion flanked by appropriate vector DNA sequences whereby recombination occurs between the corresponding regions in the recombinant DNA molecule and the vector genome. Recombinant vector progeny can now be produced in cell culture and can be selected for example genotypically or phenotypically, e.g. by hybridization,

detecting enzyme activity encoded by a gene co-integrated along with the heterologous nucleic acid sequence, or detecting the antigenic heterologous polypeptide expressed by the recombinant vector immunologically.

Next, this recombinant micro-organism can be administered to the host animal for immunization whereafter it is maintained and optionally replicates in the body of the inoculated animal, expressing in vivo a polypeptide coded for by the nucleic acid sequence according to the invention inserted in a vector organism and resulting in the stimulation of the immune system of the inoculated animal.

Suitable vectors for the incorporation of a nucleic acid sequence according to the invention can be derived from viruses such as (avian) pox viruses, e.g. vaccinia virus or fowl pox virus (EP 314,569 and WO 88/02022), herpes viruses such as HVT (WO 88/07088), adeno virus or influenza virus, or bacteria such as *E. coli* or specific *Salmonella* species. With recombinant micro-organisms of this type, the polypeptide synthesized in the host cell can be exposed as a surface antigen. In this context fusion of the said polypeptide with OMP proteins, or pilus proteins of for example *E. coli* or with synthetic provision of signal and anchor sequences which are recognized by the organism are conceivable. It is also possible that the said immunogenic polypeptide, if desired as part of a larger whole, is released inside the animal to be immunized. In all of these cases it is also possible that one or more immunogenic products will be expressed, generating protection against various pathogens and/or against various antigens of a given pathogen.

A vaccine according to the invention can be prepared by culturing a host cell infected with a vector virus comprising a nucleic acid sequence according to the invention, whereafter virus containing cells and/or vector viruses grown in the cells can be collected, optionally in a pure form, and formed to a vaccine optionally in a lyophilized form.

Above-mentioned host cells comprising a nucleic acid sequence according to the invention can also be cultured under conditions which are favourable for the expression of a polypeptide coded by said nucleic acid sequence. Vaccines may be prepared using samples of the crude culture, host cell lysates or host cell extracts, although in another embodiment more purified polypeptides according to the invention are formed to a vaccine, depending on its intended use. In order to purify the polypeptides produced, host cells containing a nucleic acid sequence according to the invention are cultured in an adequate volume and the polypeptides produced are isolated from such cells or from the medium if the protein is secreted. Polypeptides secreted into the medium can be isolated and purified by standard techniques, e.g. salt fractionation, centrifugation, ultrafiltration, chromatography, gel filtration or immuno affinity chromatography, whereas intracellular polypeptides can be isolated by first collecting said cells, disrupting the cells, for example by sonication or by other mechanically disruptive means such as French press followed by separation of the polypeptides from the other intracellular components and forming the polypeptides to a vaccine. Cell disruption could also be accomplished by chemical (e.g. EDTA treatment) or enzymatic means such as lysozyme digestion.

Antibodies or antiserum directed against a polypeptide according to the invention have potential use in passive immunotherapy, diagnostic immunoassays and generation of anti-idiotypic antibodies.

The MDV polypeptides MD18 or MD20 as described above can be used to produce antibodies, both polyclonal, monospecific and monoclonal. If polyclonal antibodies are desired, techniques for producing and processing polyclonal sera are known in the art (e.g. Mayer and Walter, eds, *Immunochemical Methods in Cell and Molecular Biology*, Academic Press, London, 1987). In short, a selected mammal, e.g. rabbit is given (multiple) injections with one of the above-mentioned immunogens, about 20 µg to about 80 µg of protein per immunization. Immunizations are given with an acceptable adjuvant, generally equal volumes of immunogen and adjuvant. Acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate or water-in-oil emulsions, with Freund's complete adjuvant being preferred for the initial immunization. Freund's incomplete adjuvant is preferred for all booster immunizations. The initial immunization consists of the administration of about 1 ml of emulsion at multiple subcutaneous sites on the backs of the rabbits. Booster immunizations utilizing an equal volume of immunogen are given at about one month intervals and are continued until adequate levels of antibodies are present in an individual rabbits serum. Blood is collected and serum isolated by methods known in the art.

Monospecific antibodies to each of the immunogens are prepared by immunizing rabbits as described above with the purified proteins and thereafter affinity purified from polyspecific antisera by a modification of the method of Hall et al. (*Nature* 311, 379-387 1984). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogeneous binding characteristics for the relevant antigen. Homogeneous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope.

Monoclonal antibody reactive against each of the MDV immunogens can be prepared by immunizing inbred mice, preferably Balb/c with the appropriate protein. The mice are immunized intraperitoneally with about 100 ng to about 10 µg immunogen per 0.5 ml in an equal volume of a suitable adjuvant. Such acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate and water-in-oil emulsions. The mice are given intravenous booster immunizations of an equal amount of the immunogen without adjuvant at about days 14, 21 and 63 post primary immunization. At about day three after the final booster immunization, individual mice are serologically tested for anti-immunogen antibodies. Spleen cells from antibody producing mice are isolated and fused with murine myeloma cells, such as SP-2/0 or the like, by techniques known in the art (Kohler and Milstein, *Nature* 256; 495-497, 1975). Hybridoma cells are selected by growth in appropriate cell culture medium such as Dulbecco's modified Eagle's medium (DMEM) containing hypoxanthine, thymidine and aminopterin in an antibody producing hybridomas are cloned, preferably using the soft agar technique of MacPherson (*Soft Agar Techniques, Tissue Culture Methods and Applications*, Kruse and Paterson, eds., Academic Press, 276, 1973). Discrete colonies are transferred into individual wells of culture plates for cultivation in an appropriate culture medium. Antibody producing cells are identified by screening with the appropriate immunogen. Immunogen positive hybridoma cells are maintained by techniques known in the art. Specific monoclonal antibodies are produced by cultivating the hybridomas in vitro or preparing ascites fluid in mice following hybridoma injection by procedures known in the art.

Anti-idiotypic antibodies are immunoglobulins which carry an "internal image" of the antigen of the pathogen against which protection is desired and can be used as an immunogen in a vaccine (Dreesman et al., *J. Infect. Disease* 151, 761, 1985). Techniques for raising anti-idiotypic antibodies are known in the art (MacNamara et al., *Science* 226, 1325, 1984).

The vaccine according to the invention can be administered in a conventional active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be prophylactically and/or therapeutically effective and immunogenic. The administration of the vaccine can be done, e.g. intradermally, subcutaneously, intramuscularly, intravenously or intranasally.

Additionally, the vaccine may also contain an aqueous medium or a water-containing suspension, often mixed with other constituents, e.g. in order to increase the activity and/or shelf life. These constituents may be salts, pH buffers, stabilizers (such as skimmed milk or casein hydrolysate), emulsifiers, adjuvants to improve the immune response (e.g. oils, muramyl dipeptide, aluminium-hydroxide, tocopherol derivatives, saponin, polyanions and amphipathic substances) and preservatives.

It is clear that a vaccine according to the invention may also contain immunogens related to other pathogens of poultry or may contain nucleic acid sequences encoding these immunogens, like antigens of Infectious Bronchitis Virus, Newcastle Disease virus, Infectious Bursal Disease virus or Marek's Disease Virus different from those disclosed herein, to produce a multivalent vaccine.

The invention also relates to an "immunochemical reagent", which reagent comprises at least one of the polypeptides according to the invention or an antigenic fragment thereof.

The term "immunochemical reagent" signifies that the polypeptides according to the invention have been bound to a suitable support or have been provided with a labelling substance.

The supports which can be used are, for example, the inner wall of a microtest well or a cuvette, a tube or capillary, a membrane, filter, test strip or the surface of a particle such as, for example, a latex particle, an erythrocyte, a dye sol, a metal sol or metal compound as sol particle.

Labelling substances which can be used are, inter alia, a radioactive isotope, a fluorescent compound, an enzyme, a dye sol, metal sol or metal compound as sol particle.

A nucleic acid sequence according to the invention can also be used to design specific probes for hybridization experiments for the detection of MDV related nucleic acids in any kind of tissue.

The present invention also provides a test kit comprising said nucleic acid sequence useful for the diagnosis of MDV infection.

The invention also relates to a test kit to be used in an immuno-assay, this test kit containing at least one immunochemical reagent according to the invention.

The immunochemical reaction which takes place using this test kit is preferably a sandwich reaction, an agglutination reaction, a competition reaction or an inhibition reaction.

For carrying out a sandwich reaction, the test kit can consist, for example, of a polypeptide according to the invention bonded to a solid support, for example the inner wall of a microtest well, and either a labelled polypeptide according to the invention or a labelled anti-antibody.



Example 1Screening of bacterial expression libraries with convalescent chicken serum

5 The vector used for establishment of the library has been  $\lambda$ gt11 (Young, R.A. and Davis, R.W. Proc. Natl. Acad. Sci. 80, 1194-1198, 1983). The genome of this expression vector contains a functional LacZ gene encoding the enzyme  $\beta$ -galactosidase with a unique EcoRI restriction site near the carboxy terminus. Insertion of DNA fragments from the MDV genome at this site, potentially results in the expression of a protein consisting of a MDV specific polypeptide fused to a major part of the  $\beta$ -galactosidase. Libraries  
 10 representing a complex range of DNA fragments can be screened at high density for recombinant clones producing a fusion protein which is recognized by an antibody probe such as the serum from MDV infected birds. The library for these experiments was made using the DNA of a pool of BamHI plasmid clones which represented about 90% of the viral genome of MDV strain GA (Fukuchi et al., J. Virol. 51, 102, 1984). DNA from these plasmids was digested with BamHI, fragmented by sonication and size-selected by centrifuga-  
 15 tion on sucrose gradients. Fragments with a size between 0.5 and 4.0 kb were isolated, tailed with dG-residues and inserted by means of a synthetic adaptor into the unique EcoRI-site of the  $\lambda$ gt10 vector (Le Bouc et al., FEBS lett. 196, 108, 1986; Huynh et al., in: Cloning Techniques, A Practical Approach, ed. Glover, D., 49-78, 1985) resulting in a library with an effective size of  $5 \times 10^4$  pfu. Phages were amplified and purified on CsCl-gradients, DNA was extracted and inserts were recovered by restriction with EcoRI.  
 20 Finally, these inserts were ligated into the EcoRI-site of the  $\lambda$ gt11 vector and recombinant phages were screened with chicken serum against MDV. This convalescent serum was obtained by infecting single-comb white leghorns (SPAFAS) with a virulent passage of the GA strain from MDV obtained from Dr. Calnek (Cornell University, NY, USA). Serum was collected over a 12 weeks period and samples were tested individually by indirect fluorescence for binding to MDV plaques in tissue cultures of chicken embryo  
 25 fibroblasts (CEF).

Six of the sera were selected based on titre and specificity. A mixture of these samples was used in a 1:100 dilution to screen the  $\lambda$ gt11 library on nitrocellulose filters according to Young, R.A. et al. (Proc. Natl. Acad. Sci. U.S.A. 82, 2583, 1985). The second antibody for incubation of the filters was an alkaline phosphatase conjugated rabbit-anti-chicken serum (Sigma, St. Louis, USA) and positive signals were  
 30 developed by the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate colour reaction (McGadey, Histochemie 23, 180, 1970). Series of candidates were selected and recombinants were plaque-purified until homogeneity. From four of these, the DNA insert in the  $\lambda$ gt11 recombinant was recovered as a EcoRI fragment and transferred to the plasmid vector pUC8 (Vieira, J. and Messing, J., Gene 19, 259, 1982) or pGEM3Z (Promega, Madison, USA). The resulting constructs were designated as pMD18, pMD20 and  
 35 pMD21.

Example 2Cloning of the MD18 gene and structural analysis of the antigen MD18

40 The gene encoding the complete amino acid sequence of antigen MD18 was isolated by screening a  $\lambda$ EMBL3 library representing the genome of MDV strain GA. The DNA used for establishment of this library was prepared by infecting chicken embryo fibroblasts (CEF) with a tissue-culture adapted passage of MD strain GA provided by Dr. Nonoyama, Showa Univ., St. Petersburg, U.S.A. Cultures were incubated until  
 45 90% cytopathic effect (CPE) had developed and total DNA was prepared by proteinase K digestion and phenol/chloroform extraction. DNA was partially digested with Sau 3A (Promega, Madison, USA) and the size fraction of about 20 kb was isolated after separation in a 0,8% agarose gel. DNA fragments were ligated with BamHI/EcoRI digested  $\lambda$ EMBL3 DNA (Promega., Madison, USA), packaged in vitro and plated on E.coli strain LE392. Screening of this library (Maniatis, T. et al., 1989, *ibid*) with the insert from pMD18  
 50 resulted among others in the isolation of  $\lambda$ GA12 containing a 21 kb DNA mapped by restriction analysis to a region of the MDV viral genome about halfway the  $U_L$  structural element. Within this 21 kb of DNA, the position of the sequence hybridizing with pMD18 was defined to a 3.8 kb BamHI fragment which was subcloned as such in both orientations using the vector pGEM3Z and resulting in the plasmids pMD41 and pMD42 respectively. Nucleotide sequence analysis in both orientations of the DNA, was performed on  
 55 progressively deleted subclones generated with the enzyme exonuclease III as described by Henikoff, S. (Gene 28, 357, 1984). After assemblage of all sequence data and translation of the sequence in the region

of interest, a primary structure was deduced for the antigen originally identified in the immuno-screening by means of the convalescent chicken serum. The complete amino acid sequence of this antigen designated MD18, is presented in SEQ ID NO: 2.

### 5 Example 3

#### Cloning of the MD20 gene and structural analysis of the antigen MD20.

Analysis directly on the insert of pMD20 and pMD21 revealed a partially overlapping nucleotide  
10 sequence suggesting that both candidates were representing the same antigen.

The gene encoding the complete amino acid sequence of this antigen was isolated by screening a  $\lambda$ EMBL3 library representing the genome of MDV strain GA. The DNA used for establishment of this library was prepared by infecting chicken embryo fibroblasts (CEF) with a tissue-culture adapted passage of MD strain GA provided by Dr. Nonoyama, Showa Univ., St. Petersburg, U.S.A. Cultures were incubated until  
15 90% cytopathic effect (CPE) had developed and total DNA was prepared by proteinase K digestion and phenol/chloroform extraction. DNA was partially digested with Sau 3A (Promega, Madison, USA) and the size fraction of about 20 kb was isolated after separation in a 0,8% agarose gel. DNA fragments were ligated with BamHI/EcoRI digested  $\lambda$ EMBL3 DNA (Promega, Madison, USA), packaged in vitro and plated on E.coli strain LE392. Screening of this library (Maniatis, T. et al., 1982, *ibid*) with the insert from pMD21  
20 resulted in the isolation of clone  $\lambda$ GA09. This clone contained a 17 kb DNA insert that is located near the junction of the  $U_L$  and  $IR_L$  in the MDV viral genome. Referring to the restriction map as published by Fukuchi et al. (J. Virol. 51, 102, 1984), the 17 kb insert included the region in between the S and  $I_2$  BamHI fragments. Restriction mapping on the DNA from  $\lambda$ GA09 and hybridization with the insert from pMD21 identified the position of the gene in a 7.5 kb SalI fragment which was subcloned as such in pGEM3Z to  
25 result in pMD26. Nucleotide sequence analysis was performed on subclones generated by both the exonuclease III treatment (Henikoff, S., Gene 28, 257, 1984) and the use of convenient restriction sites. The final sequence obtained after assembling all data from the reactions done in both orientations, was translated into the complete amino acid sequence of the antigen denominated MD20 and is shown in SEQ ID NO: 4.

### 30 Example 4

#### Insertion of the genes encoding MDV antigens MD18 and MD20 into the viral genome of herpes virus of turkey (HVT).

Based on the genome structure of HVT as published by Igarashi, T. et al. (Virology 157, 351, 1987) a region in the unique-short sequence element (Us) of the virus was selected for the insertion of foreign genes. The corresponding DNA fragment was screened from a  $\lambda$ EMBL3 library constructed by partially digesting total DNA from HVT infected CEF following a procedure used previously for MDV strain GA. The  
40 insert of one of the  $\lambda$ -isolates, characterized by the absence of any BamHI restriction site, was denominated  $\lambda$ HVT04 and analyzed in detail by physical mapping (Figure 1). The sequence present in the 17.5 kb inserted fragment represented a major part of the Us region including part of the inverted repeat structure (Igarashi, T. et al., 1987, *ibid*). One of the 1.2 kb XhoI restriction fragments from  $\lambda$ HVT04 was subcloned in pGEM3Z digested with Sal I resulting in plasmid pMD07 which contained a unique BglII site available for  
45 insertion of DNA fragments. The gene encoding antigen MD18 or MD20 was assembled from pMD41 and pMD26 respectively by removal of the excess of nucleotide sequences preceding the ATG-initiator and the creation of convenient restriction sites such as Sal I or XhoI flanking the coding region. For pMD41 this resulted in pMD46, and pMD26 gave pMD47, both restriction maps being presented in Figure 2.

A strong promoter which could direct the expression of foreign genes after their insertion into the  
50 genome of the HVT virus was selected from the LTR sequence of Rous Sarcoma Virus (RSV). The promoter has been mapped on a 580 bp NdeI/HindIII restriction fragment from pRSVcat (Gorman et al., Proc. Natl. Acad. Sci. 79, 6777, 1982) and was inserted between the HindIII and PstI sites of pGEM3Z (Promega) by means of double stranded synthetic linkers on both sides of the fragment. The connection between the HindIII site from the vector pGEM3Z and the NdeI site of the RSV fragment carrying the LTR-  
55 promoter was made with a 30 bp linker containing cohesive ends compatible with HindIII on one and NdeI on the other site. However, after ligation both restriction sites are not restored due to deliberate modifications in the outer nucleotides of the six basepair recognition sequence. In addition to the removal of these two sites, a new restriction site (BamHI) present within the linker itself was created at the corresponding

position. A second 20 bp linker was synthesized which connected the HindIII site from the LTR fragment to the PstI site from pGEM3Z, in this case without destruction of the recognition sequence on either of the ends and adding the three convenient unique restriction sites BglII, XhoI and EcoRV, to those already present in the polylinker of pGEM3Z, e.g. PstI, SalI, XhoI and BamHI. The resulting derivative of pGEM3Z, designated pVECO1, therefore contains a 650 bp restriction fragment carrying the LTR promoter sequence immediately followed by seven restriction sites available for the insertion of foreign genes. The 650 bp fragment is flanked on either end by a BamHI restriction site and has been transferred as such to the unique BglII site present in the 1,2 kb HVT insert from pMD07. The cohesive ends generated by these two restriction enzymes are compatible but ligation does not restore either of the original recognition sequences for BglII or BamHI. One of the resulting constructs, carrying the LTR in the orientation towards the TR<sub>s</sub>, was designated pVECO4 and checked by restriction mapping (Figure 3). The structure of this universal HVT recombination vector allows the insertion of foreign genes immediately downstream of the LTR promoter and subsequent integration of the complete expression cassette into the HVT genome by in vivo recombination. The positions of the different restriction sites downstream of the LTR in particular those for the enzymes BglII, XhoI and EcoRV are designed in such a way that even multiple gene insertion can be envisaged. A 2,5 kb SalI/XhoI restriction fragment derived from pMD46 carrying the MD18 gene, was inserted into the unique BglII site of pVECO4 downstream of the LTR promoter, resulting in pMD48. A 3,6 kb SalI restriction fragment derived from pMD47 carrying the MD20 gene was inserted into the unique XhoI site of pVECO4 downstream of the LTR promoter, resulting in pMD49.

DNA of the plasmids pMD48 or pMD49 was introduced together with total DNA prepared from HVT infected cells into CEF by a method based on the calcium phosphate DNA precipitation according to Graham, F and v.d. Eb, A., (Virology 52, 456, 1973) with modifications described by Morgan et al. (Avian Diseases 34, 345, 1990). Two microgram of plasmid DNA from the constructs were mixed with 15 µg of DNA from HVT infected cells in a final volume of 560 µl H<sub>2</sub>O and added to 750 µl of HBSP (20 mM KCl, 560 mM NaCl, 24 mM glucose, 3 mM Na<sub>2</sub>HPO<sub>4</sub>, 100 mM HEPES, pH 7,0). Precipitates were formed by gradually adding 190 µl of 1M CaCl<sub>2</sub> solution and incubating the mixtures at room-temperature for 30 minutes. In the meantime, 15 ml of a suspension of secondary CEF from 10 day old embryos in medium 6/B8, for which the composition is based on Glasgow's modification of Eagle's Minimal Essential Medium supplemented with 2% of foetal calf serum, were seeded in Ø 10 cm dishes at a density of 5 x 10<sup>5</sup> cells per ml. Calcium phosphate precipitated DNA was gently added to the cell suspension and dishes were incubated at 37 °C in a humidified incubator containing 5% CO<sub>2</sub> in air. After 5 hours, medium was removed and 10 ml of solution containing equal volumes of HBSP and 30% glycerol was layered onto the cells. After a one to two minute incubation, the solution was removed, cells were washed with medium 6/B8 and dishes were incubated with fresh medium for 3 to 5 days until viral CPE developed. Detection of HVT recombinants expressing the MD18 or MD20 polypeptides was done by immunofluorescence staining using specific mono- or polyvalent sera against these MDV antigens.

#### Legends

#### Figure 1

Restriction enzyme map of a DNA fragment essentially corresponding to the Us region of the HVT genome. The relative position of the insertion-region consisting of four open reading frames and non-coding sequences in between is indicated.

#### Figure 2

A. Restriction enzyme map of pMD46, containing the gene encoding MD18-antigen flanked by SalI and XhoI restriction sites. Vector plasmid was derived from pSP72 (Promega, Wisconsin, U.S.A.) by modification of the ClaI restriction site in the polylinker into SalI.

B. Restriction enzyme map of pMD47, containing the gene encoding MD20-antigen flanked by SalI restriction sites. Vector plasmid was derived from pSP72 (Promega, Wisconsin, U.S.A.) by modification of the ClaI restriction site in the polylinker into SalI.

#### Figure 3

Restriction enzyme map of pVECO4 showing the LTR-promoter inserted into the unique BglII site of the 1,2 kb XhoI HVT fragment from pMD07.

SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

(A) NAME: AKZO NV  
 (B) STREET: Velperweg 76  
 (C) CITY: Arnhem  
 (E) COUNTRY: the Netherlands  
 (F) POSTAL CODE (ZIP): 6824 BM

(ii) TITLE OF INVENTION: Recombinant Marek's disease virus

(iv) COMPUTER READABLE FORM:  
 Not Applicable

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2015 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Marek's disease herpesvirus  
 (B) STRAIN: GA

## (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 14..2005  
 (D) OTHER INFORMATION: /label= pMD18

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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    GGC TAT CTG GCA TTA TAT GGG GCG GAT GAA AGT GAT GAA TTG AAT ATC      97
    Gly Tyr Leu Ala Leu Tyr Gly Ala Asp Glu Ser Asp Glu Leu Asn Ile
       15               20               25

    GAC CGC AAA GAT ATT CGC GCC GCG ATT CCG ACA CCA GCT CCT TTA CCA     145
    Asp Arg Lys Asp Ile Arg Ala Ala Ile Pro Thr Pro Ala Pro Leu Pro
       30               35               40

45 ATA AAT ATA GAT CAC AGA AGA GAT TGC ACA GTC GGA GCA GTT CTT GCG     193
    Ile Asn Ile Asp His Arg Arg Asp Cys Thr Val Gly Ala Val Leu Ala
       45               50               55               60

```

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	65 70 75	
5	CCT GTG ATG GTA CGT ACA CTA GAG ACA GCC GCC AGT CAA GAA ATA TTC	289
	Pro Val Met Val Arg Thr Leu Glu Thr Ala Ala Ser Gln Glu Ile Phe	
	80 85 90	
10	AGC GAA CTT GAT AAT CTT AAA CCA GAT GAT AAA TTG CTA TAT ATA ATT	337
	Ser Glu Leu Asp Asn Leu Lys Pro Asp Asp Lys Leu Leu Tyr Ile Ile	
	95 100 105	
	ACA AAT TAT CTT CCA TCG GTA TCG CTG TCC TCA CGA CGC CTA GCA CCG	385
	Thr Asn Tyr Leu Pro Ser Val Ser Leu Ser Ser Arg Arg Leu Ala Pro	
	110 115 120	
15	GGG GAA ACG GCA GAT GAG ACT TTT TTG GCA CAT GTT GCT TTG TGT TTA	433
	Gly Glu Thr Ala Asp Glu Thr Phe Leu Ala His Val Ala Leu Cys Leu	
	125 130 135 140	
20	TTG GGG AAG CGA ATT GGA ACT ATT GTT ACA TAT GAT CTC ACC CCG GAA	481
	Leu Gly Lys Arg Ile Gly Thr Ile Val Thr Tyr Asp Leu Thr Pro Glu	
	145 150 155	
	GAG GCT ATA GAG CCG TTC AGA AAG CTT TCT CCA AAT TCT AAA GCG ACC	529
	Glu Ala Ile Glu Pro Phe Arg Lys Leu Ser Pro Asn Ser Lys Ala Thr	
	160 165 170	
25	TTG CTA TCA CAG GGC AAG GAA ACT GAA CGG CTC TTA GGT GAG ATG GTG	577
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30	TGG TAT CCG AGC AAA AAT GCA ATA ACC AAA GCG TTA TTA GGA ACG GCG	625
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	GTT AAT AAT ATG TTA CTG CGA GAT AGA TGG CAA ATT ATC TCC GAA CGA	673
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	CAC CCA ATT TGT GAA AAC GCA AAC CCG GGT AAC ATA CAA AAG GAT GAG	817
	His Pro Ile Cys Glu Asn Ala Asn Pro Gly Asn Ile Gln Lys Asp Glu	
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45	GAA ATG CAA GTG TGT ATC AGT CCA GCA CAA ACG AGT GAA ACG TTA AAT	865
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50		
55		

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	Ala	Gly	Val	Leu	Ser	Gly	Cys	Asn	Asp	Phe	His	Arg	Leu	Pro	His	Ser	
	285					290					295					300	
5	GAC	CCT	GCA	TCA	ACG	AGC	GAT	CAA	ACC	AAT	TTG	CAA	TCG	CTA	ATA	GAA	961
	Asp	Pro	Ala	Ser	Thr	Ser	Asp	Gln	Thr	Asn	Leu	Gln	Ser	Leu	Ile	Glu	
					305					310					315		
	CCG	TCC	ATG	AAC	ACT	CAA	TCT	TCT	CGC	CCA	CCC	GGA	GAC	GAT	TTT	ATT	1009
10	Pro	Ser	Met	Asn	Thr	Gln	Ser	Ser	Arg	Pro	Pro	Gly	Asp	Asp	Phe	Ile	
				320					325					330			
	TGG	GTC	CCG	ATT	AAA	AGC	TAT	AAT	CAG	CTA	GTA	TCG	AGA	AAT	GCT	TCT	1057
	Trp	Val	Pro	Ile	Lys	Ser	Tyr	Asn	Gln	Leu	Val	Ser	Arg	Asn	Ala	Ser	
			335					340					345				
15	CAG	CCA	ACG	AAT	ATT	CCC	GAT	ATT	GCA	ATT	ACA	TCG	AAT	CAG	CCT	CCG	1105
	Gln	Pro	Thr	Asn	Ile	Pro	Asp	Ile	Ala	Ile	Thr	Ser	Asn	Gln	Pro	Pro	
			350				355					360					
	TTT	ATT	CCC	CCG	GCG	TTA	ATG	AAT	ACA	TCG	ATA	TCA	GGT	CAA	CAC	TCC	1153
20	Phe	Ile	Pro	Pro	Ala	Leu	Met	Asn	Thr	Ser	Ile	Ser	Gly	Gln	His	Ser	
	365					370					375					380	
	ATC	CCA	AGT	GGA	TAT	GCC	CAA	TAT	GGG	TAC	CCT	ACA	CCC	GTA	GGT	ACC	1201
	Ile	Pro	Ser	Gly	Tyr	Ala	Gln	Tyr	Gly	Tyr	Pro	Thr	Pro	Val	Gly	Thr	
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25	CAT	AAC	TCT	CTG	CTT	CCA	TTG	GGA	CCT	GTA	AAT	CAA	ATG	GGC	GGA	TTT	1249
	His	Asn	Ser	Leu	Leu	Pro	Leu	Gly	Pro	Val	Asn	Gln	Met	Gly	Gly	Phe	
				400					405					410			
	CAA	TAT	GGA	CCT	CAG	GTG	TAC	CCC	TTG	TCA	TAT	GGA	CAA	TCG	CCT	TTA	1297
30	Gln	Tyr	Gly	Pro	Gln	Val	Tyr	Pro	Leu	Ser	Tyr	Gly	Gln	Ser	Pro	Leu	
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	Glu	Ala	Lys	Leu	Thr	Ala	Leu	Leu	Glu	Cys	Met	Thr	Lys	Glu	Lys	Arg	
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	CCA	GTG	GAT	GAG	GAG	CAC	AGA	GGC	GAC	GAT	ATG	CAT	ACT	ACT	AGG	GAA	1393
	Pro	Val	Asp	Glu	Glu	His	Arg	Gly	Asp	Asp	Met	His	Thr	Thr	Arg	Glu	
						450				455					460		
40	GAA	CGA	GGA	CGA	CGT	GGA	CGT	AAG	CGA	CCA	TAC	GAA	TTT	GAC	AGA	TCT	1441
	Glu	Arg	Gly	Arg	Arg	Gly	Arg	Lys	Arg	Pro	Tyr	Glu	Phe	Asp	Arg	Ser	
					465					470					475		
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50																	
55																	

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	Ala Val Thr Ser Leu Gln Lys Glu Val Glu Arg Leu Asn Gly Gly Asn	
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	CAT GCC CCA GTT TAT TAC TCA TAC CCT CCT CCG GGA ACA CAT CCC ACA	1729
	His Ala Pro Val Tyr Tyr Ser Tyr Pro Pro Pro Gly Thr His Pro Thr	
	560 565 570	
15	GTT TCA TGG CCC ATG GGA GTC GAA CGC CCT ATG CCT TCC ACG GAA GGA	1777
	Val Ser Trp Pro Met Gly Val Glu Arg Pro Met Pro Ser Thr Glu Gly	
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35		

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 663 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

50

55

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 35 40 45  
 10 His Arg Arg Asp Cys Thr Val Gly Ala Val Leu Ala Leu Ile Asp Asp  
 50 55 60  
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 85 90 95  
 20 Asn Leu Lys Pro Asp Asp Lys Leu Leu Tyr Ile Ile Thr Asn Tyr Leu  
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 130 135 140  
 Ile Gly Thr Ile Val Thr Tyr Asp Leu Thr Pro Glu Glu Ala Ile Glu  
 145 150 155 160  
 30 Pro Phe Arg Lys Leu Ser Pro Asn Ser Lys Ala Thr Leu Leu Ser Gln  
 165 170 175  
 Gly Lys Glu Thr Glu Arg Leu Leu Gly Glu Met Val Trp Tyr Pro Ser  
 180 185 190  
 35 Lys Asn Ala Ile Thr Lys Ala Leu Leu Gly Thr Ala Val Asn Asn Met  
 195 200 205  
 40 Leu Leu Arg Asp Arg Trp Gln Ile Ile Ser Glu Arg Arg Arg Met Ala  
 210 215 220  
 Gly Ile Thr Gly Gln Lys Tyr Leu Gln Ala Ser Ser Phe Thr Ala Leu  
 225 230 235 240  
 45 Thr Asp Ser Met Thr Ser Asn Asn Val Ser Val Thr His Pro Ile Cys  
 245 250 255  
 Glu Asn Ala Asn Pro Gly Asn Ile Gln Lys Asp Glu Glu Met Gln Val  
 260 265 270  
 50 Cys Ile Ser Pro Ala Gln Thr Ser Glu Thr Leu Asn Ala Gly Val Leu  
 275 280 285  
 Ser Gly Cys Asn Asp Phe His Arg Leu Pro His Ser Asp Pro Ala Ser  
 290 295 300



5 Thr Ser Asp Gln Thr Asn Leu Gln Ser Leu Ile Glu Pro Ser Met Asn  
 305 310 315 320  
 Thr Gln Ser Ser Arg Pro Pro Gly Asp Asp Phe Ile Trp Val Pro Ile  
 325 330 335  
 10 Lys Ser Tyr Asn Gln Leu Val Ser Arg Asn Ala Ser Gln Pro Thr Asn  
 340 345 350  
 Ile Pro Asp Ile Ala Ile Thr Ser Asn Gln Pro Pro Phe Ile Pro Pro  
 355 360 365  
 15 Ala Leu Met Asn Thr Ser Ile Ser Gly Gln His Ser Ile Pro Ser Gly  
 370 375 380  
 Tyr Ala Gln Tyr Gly Tyr Pro Thr Pro Val Gly Thr His Asn Ser Leu  
 385 390 395 400  
 20 Leu Pro Leu Gly Pro Val Asn Gln Met Gly Gly Phe Gln Tyr Gly Pro  
 405 410 415  
 Gln Val Tyr Pro Leu Ser Tyr Gly Gln Ser Pro Leu Glu Ala Lys Leu  
 420 425 430  
 25 Thr Ala Leu Leu Glu Cys Met Thr Lys Glu Lys Arg Pro Val Asp Glu  
 435 440 445  
 Glu His Arg Gly Asp Asp Met His Thr Thr Arg Glu Glu Arg Gly Arg  
 450 455 460  
 30 Arg Gly Arg Lys Arg Pro Tyr Glu Phe Asp Arg Ser Ile Glu Ser Asp  
 465 470 475 480  
 35 Leu Tyr Tyr Pro Gly Glu Phe Arg Arg Ser Asn Phe Ser Pro Pro Gln  
 485 490 495  
 Ala Ser Ser Met Lys Tyr Glu Glu Thr Thr Gly Gly Arg His Asp Leu  
 500 505 510  
 40 Ser Gln Thr Gly Pro Val Leu Asn Ser Leu Met Gly Ala Val Thr Ser  
 515 520 525  
 Leu Gln Lys Glu Val Glu Arg Leu Asn Gly Gly Asn Leu Pro Ile Ser  
 530 535 540  
 45 Asn Ala Gln Ser Ser Tyr Gly Val Pro Asn Gly Met His Ala Pro Val  
 545 550 555 560  
 50 Tyr Tyr Ser Tyr Pro Pro Pro Gly Thr His Pro Thr Val Ser Trp Pro  
 565 570 575  
 Met Gly Val Glu Arg Pro Met Pro Ser Thr Glu Gly Lys Thr Ser Thr  
 580 585 590

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Asn Ser Thr Val Ile Pro Val Pro Val Ser Asp Pro Glu Ala Gly Arg  
595 600 605

Asn Val Pro Ile Thr Ala Thr Ile Ser Gln Glu Arg Ser Asp Gly Ile  
610 615 620

Gln Lys Glu Ser Ile Glu Gln Ser Arg Asp Thr Met Asn Ala Ser Ala  
625 630 635 640

Val Ala Gly Ile His Arg Thr Ser Asp Ala Gly Val Asp Val Phe Ile  
645 650 655

Asn Gln Met Met Ala His Gln  
660

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3265 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Marek's disease herpesvirus
- (B) STRAIN: GA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 41..3265
- (D) OTHER INFORMATION: /label= pMD20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAGTCTTCTG TAGTCGTATG TTCTTGAGCT GGTTCGATT	ATG GCG AGA TTT TCG	55
	Met Ala Arg Phe Ser	
	1 5	
TCT ATA TCC GAT ACG CTC GAA AGT GAT GAC TCG GGA ATT AAG GTC TTA		103
Ser Ile Ser Asp Thr Leu Glu Ser Asp Asp Ser Gly Ile Lys Val Leu		
	10 15 20	
TTT GCC GTA GAT GGT TGT GCC GTG TCG TTT TCC CTG GCC CTT CTT ACA		151
Phe Ala Val Asp Gly Cys Ala Val Ser Phe Ser Leu Ala Leu Leu Thr		
	25 30 35	
GGT CAG ATA CCC TCT ACT AAC TCC GTT TAT GTT ATC GGC TAT TGG GAT		199
Gly Gln Ile Pro Ser Thr Asn Ser Val Tyr Val Ile Gly Tyr Trp Asp		
	40 45 50	
CCA AGC GAC CGA TTT TCA AGC ATA CCC TTT CTC GAC GGG GAT CCT AAT		247
Pro Ser Asp Arg Phe Ser Ser Ile Pro Phe Leu Asp Gly Asp Pro Asn		
	55 60 65	

	ACT	AAT	GAG	AGA	ATA	TCT	ACC	ACC	GTT	TGT	AAT	TTA	GAG	GAT	GTT	CCC	295
	Thr	Asn	Glu	Arg	Ile	Ser	Thr	Thr	Val	Cys	Asn	Leu	Glu	Asp	Val	Pro	
	70					75					80					85	
5	AGC	CCT	CTA	AGA	GTA	GAA	TTT	TGT	CTT	CTG	AAC	CAA	ATG	GCA	TCA	GGT	343
	Ser	Pro	Leu	Arg	Val	Glu	Phe	Cys	Leu	Leu	Asn	Gln	Met	Ala	Ser	Gly	
					90					95						100	
10	ATG	GGC	GGT	GCT	GAT	TTA	AAA	CTG	AGA	ACA	CGT	GCA	ATA	TTC	GTA	TGC	391
	Met	Gly	Gly	Ala	Asp	Leu	Lys	Leu	Arg	Thr	Arg	Ala	Ile	Phe	Val	Cys	
				105					110					115			
	CGA	TTT	ACA	TCA	TGG	TCC	GAA	ATG	AAC	GCT	ATC	GCA	AAT	TCA	ATA	ATT	439
	Arg	Phe	Thr	Ser	Trp	Ser	Glu	Met	Asn	Ala	Ile	Ala	Asn	Ser	Ile	Ile	
				120				125					130				
15	TAT	GGA	ACG	CCA	ATT	CAA	GCC	GGT	GTT	TTA	CAA	GCA	ACA	ATA	TCT	GAA	487
	Tyr	Gly	Thr	Pro	Ile	Gln	Ala	Gly	Val	Leu	Gln	Ala	Thr	Ile	Ser	Glu	
		135					140					145					
20	ACT	GAA	ACG	TTC	ATG	TTA	CAT	GAT	GAA	TTC	AAC	CTT	GCT	CTT	CAC	GTC	535
	Thr	Glu	Thr	Phe	Met	Leu	His	Asp	Glu	Phe	Asn	Leu	Ala	Leu	His	Val	
	150					155					160					165	
	TTT	CTC	AAT	GGG	TTA	TCT	CTG	AAG	GGT	CGT	AAC	AAA	AAA	GAT	GTT	TGT	583
	Phe	Leu	Asn	Gly	Leu	Ser	Leu	Lys	Gly	Arg	Asn	Lys	Lys	Asp	Val	Cys	
					170					175					180		
25	ATG	TCA	TTG	AAT	CAC	AAT	TAT	ATA	TCG	AGC	GTA	TCT	GAG	AAT	TTC	CCA	631
	Met	Ser	Leu	Asn	His	Asn	Tyr	Ile	Ser	Ser	Val	Ser	Glu	Asn	Phe	Pro	
				185					190					195			
30	AGG	GGT	AAA	CGA	GGT	CTG	ACT	GGA	CTC	TAT	TTA	CAA	CAC	GAA	CAA	AAG	679
	Arg	Gly	Lys	Arg	Gly	Leu	Thr	Gly	Leu	Tyr	Leu	Gln	His	Glu	Gln	Lys	
			200					205					210				
	GTC	ACA	GCA	GCA	TAT	CGG	CGT	ATA	TAT	GGT	GGA	TCT	ACT	ACA	ACT	GCT	727
	Val	Thr	Ala	Ala	Tyr	Arg	Arg	Ile	Tyr	Gly	Gly	Ser	Thr	Thr	Thr	Ala	
		215					220					225					
35	TTT	TGG	TAC	GTG	TCC	AAA	TTC	GGA	CCA	GAT	GAA	AAA	AGT	CTT	GTT	TTG	775
	Phe	Trp	Tyr	Val	Ser	Lys	Phe	Gly	Pro	Asp	Glu	Lys	Ser	Leu	Val	Leu	
	230					235					240					245	
40	GCC	CTA	CGT	TAT	TAC	CTT	TTG	CAG	GCA	CAG	GAA	GAA	GTT	ACT	GGT	ATT	823
	Ala	Leu	Arg	Tyr	Tyr	Leu	Leu	Gln	Ala	Gln	Glu	Glu	Val	Thr	Gly	Ile	
					250					255					260		
	GCA	ACA	GGC	TAT	GAT	CTG	CAA	GCC	ATA	AAA	GAT	ATA	TGC	AAA	ACA	TAC	871
	Ala	Thr	Gly	Tyr	Asp	Leu	Gln	Ala	Ile	Lys	Asp	Ile	Cys	Lys	Thr	Tyr	
				265					270					275			
45	GCA	GTG	TCG	GTA	AAT	CCC	AAT	CCC	ACG	GGA	TTT	TTG	GCT	GCC	GAT	TTA	919
	Ala	Val	Ser	Val	Asn	Pro	Asn	Pro	Thr	Gly	Phe	Leu	Ala	Ala	Asp	Leu	
			280					285					290				
50																	
55																	

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	ACG	TCA	TTT	AGT	AGA	TTA	TCA	CGT	TTT	TGT	TGT	TTA	AGT	TAC	TAT	TCC	967
	Thr	Ser	Phe	Ser	Arg	Leu	Ser	Arg	Phe	Cys	Cys	Leu	Ser	Tyr	Tyr	Ser	
	295						300					305					
5	AAA	GGC	TCT	GTG	GCC	ATA	GCA	TTT	CCA	TCA	TAT	GTG	GAA	CGC	AGG	ATT	1015
	Lys	Gly	Ser	Val	Ala	Ile	Ala	Phe	Pro	Ser	Tyr	Val	Glu	Arg	Arg	Ile	
	310					315					320					325	
	ATG	GCC	GAT	ATC	GCA	GAA	GTG	GAT	GCA	TTG	AGA	GAA	TAT	ATA	GAA	AGA	1063
10	Met	Ala	Asp	Ile	Ala	Glu	Val	Asp	Ala	Leu	Arg	Glu	Tyr	Ile	Glu	Arg	
					330					335					340		
	GAC	AGA	CCC	AGT	TTG	AAG	ATT	TCG	GAT	TTG	GAA	TTC	GTT	AAA	TAT	ATA	1111
	Asp	Arg	Pro	Ser	Leu	Lys	Ile	Ser	Asp	Leu	Glu	Phe	Val	Lys	Tyr	Ile	
				345				350					355				
15	TAT	TTA	GCT	TAT	TTT	GAA	TGT	TAT	AAC	CGC	GAA	CAG	TTA	AAA	CGA	CAT	1159
	Tyr	Leu	Ala	Tyr	Phe	Glu	Cys	Tyr	Asn	Arg	Glu	Gln	Leu	Lys	Arg	His	
			360					365					370				
	TTG	AAA	GAT	GTG	ACA	GTA	AGT	TTG	CCC	GAT	GAA	GAC	ATT	TAC	AAG	AAG	1207
20	Leu	Lys	Asp	Val	Thr	Val	Ser	Leu	Pro	Asp	Glu	Asp	Ile	Tyr	Lys	Lys	
		375					380					385					
	TCT	TCA	CTA	GGC	AAG	TGT	GCA	GTA	GAA	AAT	TTT	TTT	ACA	CAT	GTG	AGA	1255
	Ser	Ser	Leu	Gly	Lys	Cys	Ala	Val	Glu	Asn	Phe	Phe	Thr	His	Val	Arg	
						395					400					405	
25	TCT	AGA	TTG	AAC	GTG	AAT	GAC	CAC	ATA	GCC	CAT	AAT	GTA	TTG	CCC	GAA	1303
	Ser	Arg	Leu	Asn	Val	Asn	Asp	His	Ile	Ala	His	Asn	Val	Leu	Pro	Glu	
					410					415					420		
	CAA	GTA	GAA	ATG	GGA	AAT	AAG	CTA	GTC	CGA	AAG	TTT	GGA	CGT	GCC	AGA	1351
30	Gln	Val	Glu	Met	Gly	Asn	Lys	Leu	Val	Arg	Lys	Phe	Gly	Arg	Ala	Arg	
				425					430					435			
	ATG	TAT	CTG	TCA	ACT	ACG	ATG	ACT	AAC	GAG	TCG	CAC	TTC	ACT	GGA	ATA	1399
	Met	Tyr	Leu	Ser	Thr	Thr	Met	Thr	Asn	Glu	Ser	His	Phe	Thr	Gly	Ile	
				440				445					450				
35	TGT	GAA	TGT	GCA	TCT	GTG	ATT	TTA	AAG	CGA	CTG	GAC	ACT	CTA	GAA	ATG	1447
	Cys	Glu	Cys	Ala	Ser	Val	Ile	Leu	Lys	Arg	Leu	Asp	Thr	Leu	Glu	Met	
		455					460					465					
	AAA	TTG	CAA	AAG	TAT	GGT	TGG	CCG	TCT	GAT	CGT	GTG	GAT	GGT	TCC	AAT	1495
40	Lys	Leu	Gln	Lys	Tyr	Gly	Trp	Pro	Ser	Asp	Arg	Val	Asp	Gly	Ser	Asn	
		470				475					480					485	
	CTA	ATG	GCC	GAT	AAT	CAG	AAC	AAC	TCT	ACT	TTA	ATA	CCG	TAT	GAT	AAA	1543
	Leu	Met	Ala	Asp	Asn	Gln	Asn	Asn	Ser	Thr	Leu	Ile	Pro	Tyr	Asp	Lys	
					490					495					500		
45	TCT	AGG	TCT	TCT	GGA	ATG	ATA	CTC	GAG	TGT	TCG	AAC	ACT	CAT	TCT	CGA	1591
	Ser	Arg	Ser	Ser	Gly	Met	Ile	Leu	Glu	Cys	Ser	Asn	Thr	His	Ser	Arg	
				505					510					515			
50																	
55																	

	GGG GGG CCG ATG ATA GTT AAA AGG TTA TTA GCT TTA GTA TCT GCC GAT	1639
	Gly Gly Pro Met Ile Val Lys Arg Leu Leu Ala Leu Val Ser Ala Asp	
	520 525 530	
5	TCT CGC GCA GGG GGA ATC GGC CCA GCT AAC ATG CTC ATG GGG ATT GAC	1687
	Ser Arg Ala Gly Gly Ile Gly Pro Ala Asn Met Leu Met Gly Ile Asp	
	535 540 545	
10	TCT GCA ATA GAT GGA CCC CTT CCA GTT TAC CGT GTG GGC ATG TCA AAG	1735
	Ser Ala Ile Asp Gly Pro Leu Pro Val Tyr Arg Val Gly Met Ser Lys	
	550 555 560 565	
	GGC AGA CAG GCT TTT ACG GTG CTT ATG ACC GAA TGT TGG GAA AGG ACC	1783
	Gly Arg Gln Ala Phe Thr Val Leu Met Thr Glu Cys Trp Glu Arg Thr	
	570 575 580	
15	ATT CCA TCT CCG GGA AGT GCG AAA GCG CAT TTG ATC AAG CTT AAC AAC	1831
	Ile Pro Ser Pro Gly Ser Ala Lys Ala His Leu Ile Lys Leu Asn Asn	
	585 590 595	
20	TCT TAC GGT ACT TCG ACA GAA GAC TTG ATT TCA CGA GAC TTA TTC CTA	1879
	Ser Tyr Gly Thr Ser Thr Glu Asp Leu Ile Ser Arg Asp Leu Phe Leu	
	600 605 610	
	ACT TCT GAA ATC GAA CAG CTT ATC GGA AGC ACA GTA GAA TTG CCG GAG	1927
	Thr Ser Glu Ile Glu Gln Leu Ile Gly Ser Thr Val Glu Leu Pro Glu	
	615 620 625	
25	ATT ACA TGT GGC TCT GCC GAT GAA CAG CAA TAT ATA AAC CGC AAT GAA	1975
	Ile Thr Cys Gly Ser Ala Asp Glu Gln Gln Tyr Ile Asn Arg Asn Glu	
	630 635 640 645	
30	GTC TTT AAT GGG AAT CTT GCG ATA GGA AAT ATA GTT TTA GAT GTG GAT	2023
	Val Phe Asn Gly Asn Leu Ala Ile Gly Asn Ile Val Leu Asp Val Asp	
	650 655 660	
	ATA CAT TTA AGA AAC CCC ATA CCT CTT AGA CTT ATG CAT GCA GCG ATA	2071
	Ile His Leu Arg Asn Pro Ile Pro Leu Arg Leu Met His Ala Ala Ile	
	665 670 675	
35	CGA GGT TTT AGA AGT GGT ATA CTC AGA GCT TTG GCC TTA TTG CTA CCA	2119
	Arg Gly Phe Arg Ser Gly Ile Leu Arg Ala Leu Ala Leu Leu Leu Pro	
	680 685 690	
40	AAG GCA AAT ATC GAC CAT GGC TCA TAC CCG TGT TAC TTT TAT AAG AGT	2167
	Lys Ala Asn Ile Asp His Gly Ser Tyr Pro Cys Tyr Phe Tyr Lys Ser	
	695 700 705	
	TCG TGC AAG AAA TCT AGA GTA ATG GGG GGA GCG CCT TGG ATG CTC CAT	2215
	Ser Cys Lys Lys Ser Arg Val Met Gly Gly Ala Pro Trp Met Leu His	
	710 715 720 725	
45	GAT GCA GAA CTT GCC CCA GAT TAT TCG ATG TTT GAA AAT GCG GAG TTT	2263
	Asp Ala Glu Leu Ala Pro Asp Tyr Ser Met Phe Glu Asn Ala Glu Phe	
	730 735 740	

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	GAT	TTA	GAA	ATG	GGC	ATA	GAT	GAC	CCT	TTA	CTC	ATA	GAC	CAA	ATA	GAT	2311
	Asp	Leu	Glu	Met	Gly	Ile	Asp	Asp	Pro	Leu	Leu	Ile	Asp	Gln	Ile	Asp	
				745					750					755			
5	GAA	TCT	CTT	ACT	AGA	TGG	AGC	TCA	GAA	TCA	TCA	AGG	AGT	GTC	GAT	TTG	2359
	Glu	Ser	Leu	Thr	Arg	Trp	Ser	Ser	Glu	Ser	Ser	Arg	Ser	Val	Asp	Leu	
			760					765					770				
	GAT	CCA	GAT	AAG	CCA	TGC	GGT	TGC	CAT	GAT	AAA	ATC	GGA	TTG	AGG	GTT	2407
10	Asp	Pro	Asp	Lys	Pro	Cys	Gly	Cys	His	Asp	Lys	Ile	Gly	Leu	Arg	Val	
		775					780					785					
	TGC	ATT	CCA	GTA	CCC	TCT	CCA	TAT	TTA	CTT	GTG	GGT	AGC	AAG	ACA	TTG	2455
	Cys	Ile	Pro	Val	Pro	Ser	Pro	Tyr	Leu	Leu	Val	Gly	Ser	Lys	Thr	Leu	
		790				795					800					805	
15	GCC	GGA	TTG	TCT	CGA	ATC	ATT	CAA	CAA	GCC	GTG	CTC	TTA	GAG	CGC	AAT	2503
	Ala	Gly	Leu	Ser	Arg	Ile	Ile	Gln	Gln	Ala	Val	Leu	Leu	Glu	Arg	Asn	
					810					815					820		
	TTT	GTA	GAA	ACT	ATA	GGG	CCA	TAT	CTG	AAA	AAT	TAT	GAG	ATA	ATT	GAT	2551
20	Phe	Val	Glu	Thr	Ile	Gly	Pro	Tyr	Leu	Lys	Asn	Tyr	Glu	Ile	Ile	Asp	
				825					830					835			
	AGT	GGC	GTA	TAT	GGT	CAT	GGG	CGT	AGC	TTA	CGT	CTG	CCG	TTT	TTT	GGC	2599
	Ser	Gly	Val	Tyr	Gly	His	Gly	Arg	Ser	Leu	Arg	Leu	Pro	Phe	Phe	Gly	
			840					845					850				
25	AAA	ATT	GAT	GAA	AAC	GGT	ATC	GTG	TCT	AGA	AGA	CTT	GTA	CCG	TTT	TTC	2647
	Lys	Ile	Asp	Glu	Asn	Gly	Ile	Val	Ser	Arg	Arg	Leu	Val	Pro	Phe	Phe	
		855					860					865					
	GTG	ATA	CCA	GAT	GAT	TGT	GCT	GAC	ATG	GAG	AAG	TTT	ATT	GTG	GCC	CAT	2695
30	Val	Ile	Pro	Asp	Asp	Cys	Ala	Asp	Met	Glu	Lys	Phe	Ile	Val	Ala	His	
		870				875					880					885	
	TTC	GAA	CCT	AAA	AAC	TTC	CAT	TTT	CAC	AGC	TCT	ATC	CCG	CTA	GAA	AAG	2743
	Phe	Glu	Pro	Lys	Asn	Phe	His	Phe	His	Ser	Ser	Ile	Pro	Leu	Glu	Lys	
				890						895					900		
35	GCC	GCC	ATA	ATT	CTG	AAA	GAT	ATA	GGT	GGC	GAA	TAT	GCA	GGT	TTC	TTC	2791
	Ala	Ala	Ile	Ile	Leu	Lys	Asp	Ile	Gly	Gly	Glu	Tyr	Ala	Gly	Phe	Phe	
			905					910						915			
	GAA	AGA	AAA	ATT	ACA	GTA	AAT	AGA	GAT	ATA	TTT	TTC	GGG	ACT	CGA	TTA	2839
40	Glu	Arg	Lys	Ile	Thr	Val	Asn	Arg	Asp	Ile	Phe	Phe	Gly	Thr	Arg	Leu	
			920					925					930				
	TCT	TTA	TCA	ATA	GCT	CTC	AGG	GAA	AGG	GGG	GTA	GAT	ATA	AAT	GAT	TGT	2887
	Ser	Leu	Ser	Ile	Ala	Leu	Arg	Glu	Arg	Gly	Val	Asp	Ile	Asn	Asp	Cys	
		935					940					945					
45	GCT	GCC	ATT	ACA	ACA	TTT	GTA	ACA	GAT	CAC	ATT	TTA	GAT	GAT	ATT	ATA	2935
	Ala	Ala	Ile	Thr	Thr	Phe	Val	Thr	Asp	His	Ile	Leu	Asp	Asp	Ile	Ile	
		950				955					960					965	

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ACA TAC GTA TAT GAG CAT ATA CCA GAT CAC GCA ATC GAA TAT CAA AAT 2983  
 Thr Tyr Val Tyr Glu His Ile Pro Asp His Ala Ile Glu Tyr Gln Asn  
 970 975 980  
 5 CTT TCT GTC TCG TGT TGT GTT GTC AAA TCG GAT TGG ATC CTG CTG CAG 3031  
 Leu Ser Val Ser Cys Cys Val Val Lys Ser Asp Trp Ile Leu Leu Gln  
 985 990 995  
 CTA ATC CCC AAT AAA ACA ATA GGA TAT CGT CAC GGG TTT ACA TGT GTG 3079  
 Leu Ile Pro Asn Lys Thr Ile Gly Tyr Arg His Gly Phe Thr Cys Val  
 1000 1005 1010  
 10 AGA TTT AAG CAT GCA AGA GCA AGG CGA GCG AGT GCA CGT TCT TAT TTG 3127  
 Arg Phe Lys His Ala Arg Ala Arg Arg Ala Ser Ala Arg Ser Tyr Leu  
 1015 1020 1025  
 15 GCT CTG AAC GTC GAT GCG CAT GGT AGG TTG TGC GTA TGT GTA ATT CAA 3175  
 Ala Leu Asn Val Asp Ala His Gly Arg Leu Cys Val Cys Val Ile Gln  
 1030 1035 1040 1045  
 CAG TGT TTT GCG GCC AAG TGC GGA AAT AAT AAA CTT CGC ACA CTT TTC 3223  
 Gln Cys Phe Ala Ala Lys Cys Gly Asn Asn Lys Leu Arg Thr Leu Phe  
 1050 1055 1060  
 20 ACG GTA GAT ATT GAC TCG AAA TGT CGA TTA GAA CAT CAA TAG 3265  
 Thr Val Asp Ile Asp Ser Lys Cys Arg Leu Glu His Gln  
 1065 1070

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1074 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

35

Met Ala Arg Phe Ser Ser Ile Ser Asp Thr Leu Glu Ser Asp Asp Ser  
 1 5 10 15

Gly Ile Lys Val Leu Phe Ala Val Asp Gly Cys Ala Val Ser Phe Ser  
 20 25 30

40

Leu Ala Leu Leu Thr Gly Gln Ile Pro Ser Thr Asn Ser Val Tyr Val  
 35 40 45

Ile Gly Tyr Trp Asp Pro Ser Asp Arg Phe Ser Ser Ile Pro Phe Leu  
 50 55 60

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Asp Gly Asp Pro Asn Thr Asn Glu Arg Ile Ser Thr Thr Val Cys Asn  
 65 70 75 80

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	Leu	Glu	Asp	Val	Pro	Ser	Pro	Leu	Arg	Val	Glu	Phe	Cys	Leu	Leu	Asn
					85					90					95	
5	Gln	Met	Ala	Ser	Gly	Met	Gly	Gly	Ala	Asp	Leu	Lys	Leu	Arg	Thr	Arg
				100					105					110		
	Ala	Ile	Phe	Val	Cys	Arg	Phe	Thr	Ser	Trp	Ser	Glu	Met	Asn	Ala	Ile
			115					120					125			
10	Ala	Asn	Ser	Ile	Ile	Tyr	Gly	Thr	Pro	Ile	Gln	Ala	Gly	Val	Leu	Gln
		130					135					140				
	Ala	Thr	Ile	Ser	Glu	Thr	Glu	Thr	Phe	Met	Leu	His	Asp	Glu	Phe	Asn
15		145				150					155					160
	Leu	Ala	Leu	His	Val	Phe	Leu	Asn	Gly	Leu	Ser	Leu	Lys	Gly	Arg	Asn
					165					170					175	
20	Lys	Lys	Asp	Val	Cys	Met	Ser	Leu	Asn	His	Asn	Tyr	Ile	Ser	Ser	Val
				180					185					190		
	Ser	Glu	Asn	Phe	Pro	Arg	Gly	Lys	Arg	Gly	Leu	Thr	Gly	Leu	Tyr	Leu
			195					200					205			
25	Gln	His	Glu	Gln	Lys	Val	Thr	Ala	Ala	Tyr	Arg	Arg	Ile	Tyr	Gly	Gly
		210					215					220				
	Ser	Thr	Thr	Thr	Ala	Phe	Trp	Tyr	Val	Ser	Lys	Phe	Gly	Pro	Asp	Glu
		225				230					235					240
30	Lys	Ser	Leu	Val	Leu	Ala	Leu	Arg	Tyr	Tyr	Leu	Leu	Gln	Ala	Gln	Glu
					245					250					255	
	Glu	Val	Thr	Gly	Ile	Ala	Thr	Gly	Tyr	Asp	Leu	Gln	Ala	Ile	Lys	Asp
35				260					265						270	
	Ile	Cys	Lys	Thr	Tyr	Ala	Val	Ser	Val	Asn	Pro	Asn	Pro	Thr	Gly	Phe
			275					280					285			
40	Leu	Ala	Ala	Asp	Leu	Thr	Ser	Phe	Ser	Arg	Leu	Ser	Arg	Phe	Cys	Cys
		290					295					300				
	Leu	Ser	Tyr	Tyr	Ser	Lys	Gly	Ser	Val	Ala	Ile	Ala	Phe	Pro	Ser	Tyr
		305				310					315					320
45	Val	Glu	Arg	Arg	Ile	Met	Ala	Asp	Ile	Ala	Glu	Val	Asp	Ala	Leu	Arg
					325					330					335	
	Glu	Tyr	Ile	Glu	Arg	Asp	Arg	Pro	Ser	Leu	Lys	Ile	Ser	Asp	Leu	Glu
				340					345					350		
50	Phe	Val	Lys	Tyr	Ile	Tyr	Leu	Ala	Tyr	Phe	Glu	Cys	Tyr	Asn	Arg	Glu
			355					360					365			
	Gln	Leu	Lys	Arg	His	Leu	Lys	Asp	Val	Thr	Val	Ser	Leu	Pro	Asp	Glu
55		370					375					380				



5 Asp Ile Tyr Lys Lys Ser Ser Leu Gly Lys Cys Ala Val Glu Asn Phe  
 385 390 395 400  
 Phe Thr His Val Arg Ser Arg Leu Asn Val Asn Asp His Ile Ala His  
 405 410 415  
 10 Asn Val Leu Pro Glu Gln Val Glu Met Gly Asn Lys Leu Val Arg Lys  
 420 425 430  
 Phe Gly Arg Ala Arg Met Tyr Leu Ser Thr Thr Met Thr Asn Glu Ser  
 435 440 445  
 15 His Phe Thr Gly Ile Cys Glu Cys Ala Ser Val Ile Leu Lys Arg Leu  
 450 455 460  
 Asp Thr Leu Glu Met Lys Leu Gln Lys Tyr Gly Trp Pro Ser Asp Arg  
 465 470 475 480  
 20 Val Asp Gly Ser Asn Leu Met Ala Asp Asn Gln Asn Asn Ser Thr Leu  
 485 490 495  
 Ile Pro Tyr Asp Lys Ser Arg Ser Ser Gly Met Ile Leu Glu Cys Ser  
 500 505 510  
 25 Asn Thr His Ser Arg Gly Gly Pro Met Ile Val Lys Arg Leu Leu Ala  
 515 520 525  
 Leu Val Ser Ala Asp Ser Arg Ala Gly Gly Ile Gly Pro Ala Asn Met  
 530 535 540  
 30 Leu Met Gly Ile Asp Ser Ala Ile Asp Gly Pro Leu Pro Val Tyr Arg  
 545 550 555 560  
 35 Val Gly Met Ser Lys Gly Arg Gln Ala Phe Thr Val Leu Met Thr Glu  
 565 570 575  
 Cys Trp Glu Arg Thr Ile Pro Ser Pro Gly Ser Ala Lys Ala His Leu  
 580 585 590  
 40 Ile Lys Leu Asn Asn Ser Tyr Gly Thr Ser Thr Glu Asp Leu Ile Ser  
 595 600 605  
 Arg Asp Leu Phe Leu Thr Ser Glu Ile Glu Gln Leu Ile Gly Ser Thr  
 610 615 620  
 45 Val Glu Leu Pro Glu Ile Thr Cys Gly Ser Ala Asp Glu Gln Gln Tyr  
 625 630 635 640  
 Ile Asn Arg Asn Glu Val Phe Asn Gly Asn Leu Ala Ile Gly Asn Ile  
 645 650 655  
 50 Val Leu Asp Val Asp Ile His Leu Arg Asn Pro Ile Pro Leu Arg Leu  
 660 665 670

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Met His Ala Ala Ile Arg Gly Phe Arg Ser Gly Ile Leu Arg Ala Leu  
 675 680 685  
 5  
 Ala Leu Leu Leu Pro Lys Ala Asn Ile Asp His Gly Ser Tyr Pro Cys  
 690 695 700  
 Tyr Phe Tyr Lys Ser Ser Cys Lys Lys Ser Arg Val Met Gly Gly Ala  
 705 710 715 720  
 10  
 Pro Trp Met Leu His Asp Ala Glu Leu Ala Pro Asp Tyr Ser Met Phe  
 725 730 735  
 Glu Asn Ala Glu Phe Asp Leu Glu Met Gly Ile Asp Asp Pro Leu Leu  
 740 745 750  
 15  
 Ile Asp Gln Ile Asp Glu Ser Leu Thr Arg Trp Ser Ser Glu Ser Ser  
 755 760 765  
 Arg Ser Val Asp Leu Asp Pro Asp Lys Pro Cys Gly Cys His Asp Lys  
 770 775 780  
 20  
 Ile Gly Leu Arg Val Cys Ile Pro Val Pro Ser Pro Tyr Leu Leu Val  
 785 790 795 800  
 25  
 Gly Ser Lys Thr Leu Ala Gly Leu Ser Arg Ile Ile Gln Gln Ala Val  
 805 810 815  
 Leu Leu Glu Arg Asn Phe Val Glu Thr Ile Gly Pro Tyr Leu Lys Asn  
 820 825 830  
 30  
 Tyr Glu Ile Ile Asp Ser Gly Val Tyr Gly His Gly Arg Ser Leu Arg  
 835 840 845  
 Leu Pro Phe Phe Gly Lys Ile Asp Glu Asn Gly Ile Val Ser Arg Arg  
 850 855 860  
 35  
 Leu Val Pro Phe Phe Val Ile Pro Asp Asp Cys Ala Asp Met Glu Lys  
 865 870 875 880  
 Phe Ile Val Ala His Phe Glu Pro Lys Asn Phe His Phe His Ser Ser  
 885 890 895  
 40  
 Ile Pro Leu Glu Lys Ala Ala Ile Ile Leu Lys Asp Ile Gly Gly Glu  
 900 905 910  
 45  
 Tyr Ala Gly Phe Phe Glu Arg Lys Ile Thr Val Asn Arg Asp Ile Phe  
 915 920 925  
 Phe Gly Thr Arg Leu Ser Leu Ser Ile Ala Leu Arg Glu Arg Gly Val  
 930 935 940  
 50  
 Asp Ile Asn Asp Cys Ala Ala Ile Thr Thr Phe Val Thr Asp His Ile  
 945 950 955 960  
 Leu Asp Asp Ile Ile Thr Tyr Val Tyr Glu His Ile Pro Asp His Ala  
 965 970 975  
 55

5	Ile Glu Tyr Gln Asn Leu Ser Val Ser Cys Cys Val Val Lys Ser Asp 980 985 990
	Trp Ile Leu Leu Gln Leu Ile Pro Asn Lys Thr Ile Gly Tyr Arg His 995 1000 1005
10	Gly Phe Thr Cys Val Arg Phe Lys His Ala Arg Ala Arg Arg Ala Ser 1010 1015 1020
	Ala Arg Ser Tyr Leu Ala Leu Asn Val Asp Ala His Gly Arg Leu Cys 1025 1030 1035 1040
15	Val Cys Val Ile Gln Gln Cys Phe Ala Ala Lys Cys Gly Asn Asn Lys 1045 1050 1055
	Leu Arg Thr Leu Phe Thr Val Asp Ile Asp Ser Lys Cys Arg Leu Glu 1060 1065 1070
20	His Gln 1074

## Claims

1. A nucleic acid sequence encoding a MDV polypeptide characterized in that said sequence encodes at least a part of a polypeptide selected from the group consisting of:
  - a MD18 having an amino acid sequence shown in SEQ ID NO:2
  - b MD20 having an amino acid sequence shown in SEQ ID NO:4and functional equivalents thereof.
2. A nucleic acid sequence according to claim 1, characterized in that said sequence at least contains a part of the deoxyribonucleic acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 3
3. A recombinant nucleic acid molecule comprising a nucleic acid sequence according to claims 1 or 2.
4. A recombinant nucleic acid molecule according to claim 3, characterized in that the nucleic acid sequence is operably linked to an expression control sequence.
5. A vector virus containing a nucleic acid sequence according to claims 1 or 2.
6. A host cell transformed with a nucleic acid sequence according to claims 1-2 or a recombinant nucleic acid molecule according to claims 3-4 or containing a vector virus according to claim 5.
7. A polypeptide displaying MDV immunological characteristics containing at least part of the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4 or functional equivalents thereof.
8. A MDV polypeptide encoded by a nucleic acid sequence according to claims 1-2.
9. An antibody or antiserum immunoreactive with a polypeptide according to claims 7 or 8.
10. A vaccine for the protection of poultry against MDV infection, characterized in that it comprises a recombinant nucleic acid molecule according to claim 4, a vector virus according to claim 5, a host cell according to claim 6 or a polypeptide according to claims 7-8.

11. A method for preparation of MDV vaccine, characterized in that a host cell according to claim 6 is cultured, whereafter MDV containing material is collected and processed to a pharmaceutical preparation with immunizing activity.

5 12. A method for the preparation of an MDV vaccine, characterized in that a polypeptide according to claims 7-8 is processed to a pharmaceutical preparation with immunizing activity.

13. A method for the protection of poultry against MDV infection characterized in that an effective amount of a vaccine according to claim 10 is administered to the animals.

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Fig 1

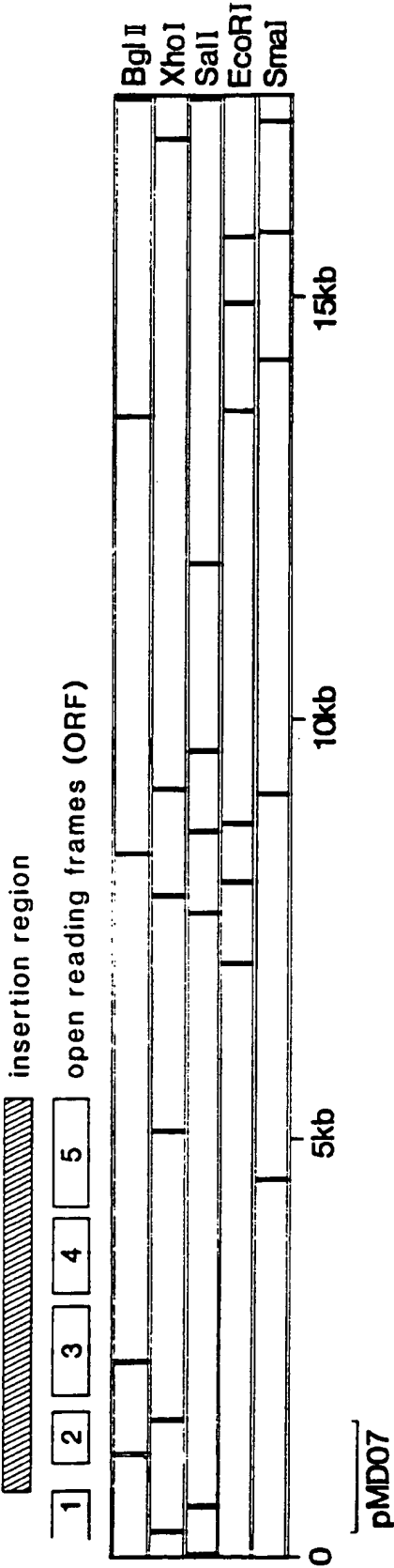


Fig 2

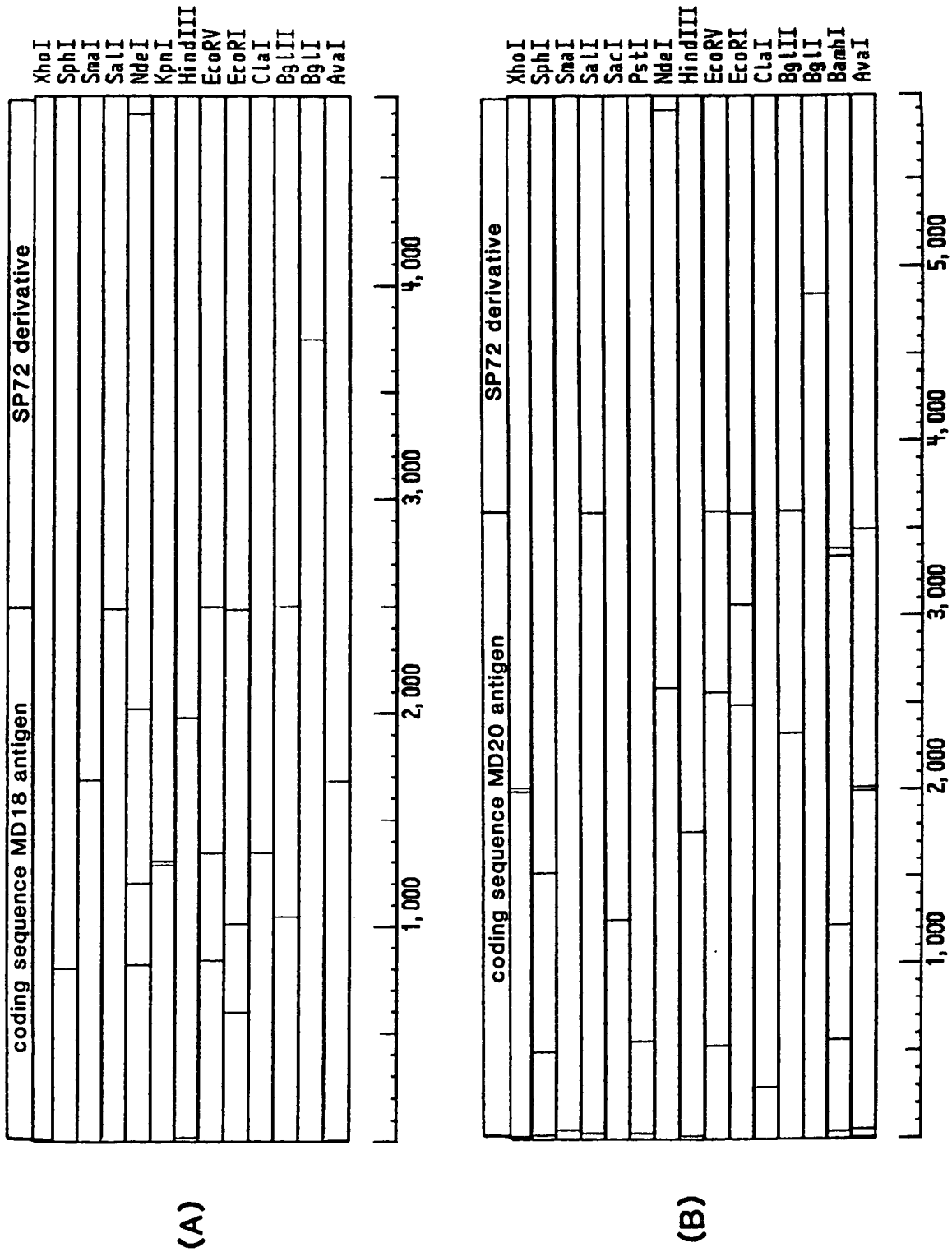
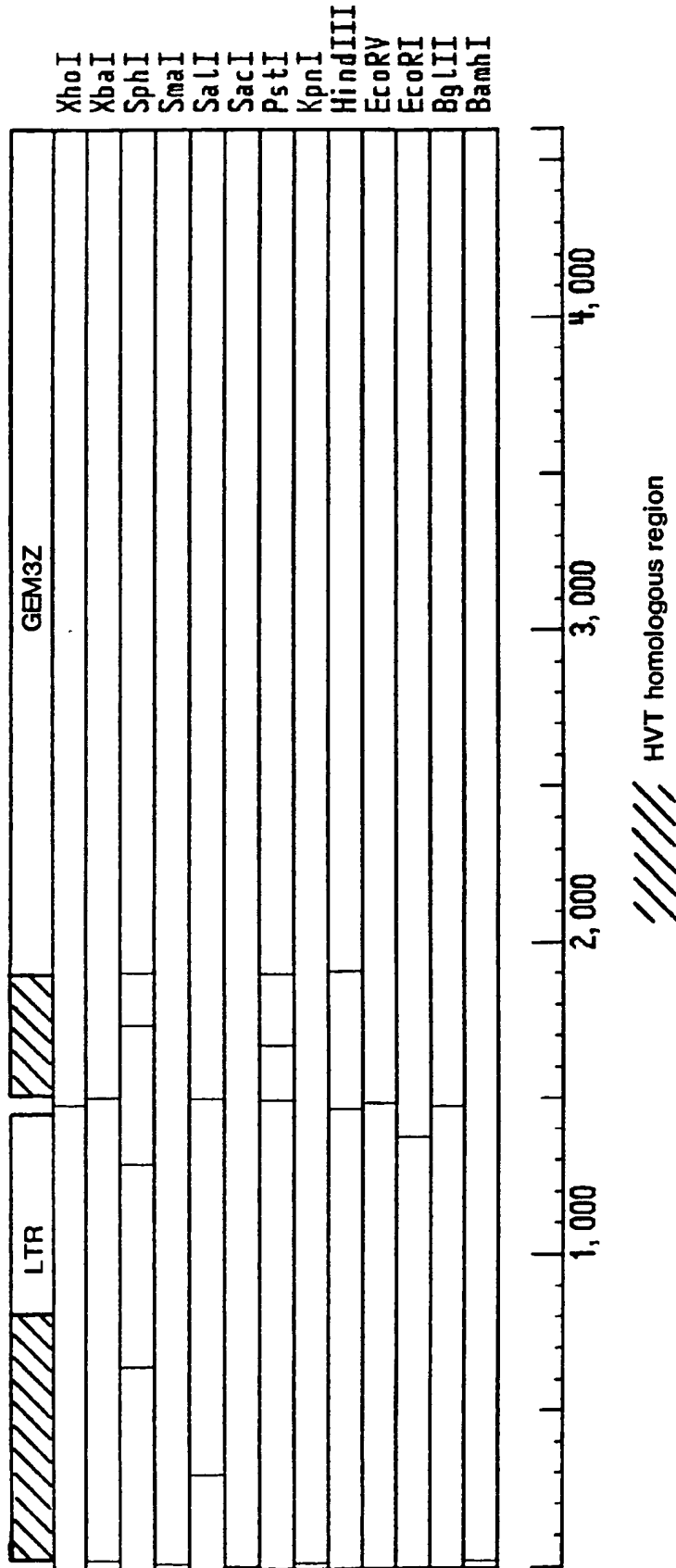


Fig 3



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54 **Marek's disease virus vaccine.**

57 The invention is concerned with the MD18 and MD20 polypeptides of Marek's Disease virus which can be used to vaccinate poultry against MD.

The invention also relates to nucleic acid sequences encoding the MD18 or MD20 polypeptides. Said sequences can be used for the preparation of a subunit or vector vaccine.

**EP 0 486 106 A3**





European Patent  
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# PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

Application Number

EP 91 20 2947  
Page 1

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
A	WO-A-9 002 803 (RHONE-MERIEUX) 22 March 1990 * the whole document * ---	1-13	C12N15/38 C12N15/86 C12N5/10 A61K39/255 A61K39/395 C12P21/00
A	J.GEN.VIROL. vol. 69, 1988, pages 2033 - 2042 BUCKMASTER, A.E., ET AL. 'Gene sequence and mapping data from Marek's Disease Virus and Herpesvirus of Turkeys: implications for herpesvirus classification' * the whole document * ---	1-13	
A	J.GEN.VIROL. vol. 69, 1988, pages 1531 - 1574 MCGOECH, D.J., ET AL. 'The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1' * page 1561 UL26 * --- -/--	1-13	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			C12N A61K C12P
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search THE HAGUE		Date of completion of the search 15 OCTOBER 1992	Examiner MADDOX A.D.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- A : technological background O : non-written disclosure P : intermediate document A : member of the same patent family, corresponding document	



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	BIOLOGICAL ABSTRACTS BR35:65580 * abstract * & J. AM. VET. MED. ASSOC.; 125TH ANNUAL AMERICAN VETERINARY MEDICAL ASSOCIATION MEETING, JULY 17-26, 1988. vol. 192, no. 12, 1988, page 1789 CUI, Z., ET AL. 'Marek's disease virus gene clones constructed in bacteriophage and identified with monoclonal antibodies' ---	9	
A	J. IMMUNOL. vol. 130, no. 2, February 1983, pages 1003 - 1006 LEE, L.F., ET AL. 'Monoclonal antibodies with specificity for three different serotypes of Marek's Disease Virus in chickens' * the whole document * -----	9	TECHNICAL FIELDS SEARCHED (Int. Cl. 5)



EP 91 202 947 -C-

Remark: Although claim 13 is directed to a method of treatment of the animal body (Art. 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition.